prejudice. New claims 42 and 43 have been added. Support for claim 42 is found in claim 1 as originally filed. Support for claim 43 is found in claims 1 and 20 as originally filed. Claims 1 and 25 have been amended to recite "a complementarity-determining region that recognizes a metal chelate or portions thereof." Support for this amendment is found in the specification at page 11, lines 20-22. Claim 18 has been amended to delete the recitation "that may contain one or more sites." Claim 10 has been amended ***solely*** to correct a typographical error. As amended, claim 10 recites "SEQ ID NO:5" and "FIG. 12." Support for this amendment is found in the specification at page 8, lines 20-21. No new matter has been introduced by these amendments.

For the convenience of the Examiner, a marked-up version of the changes made to the claims by the present Amendment is attached as Appendix A. In addition, all of the pending claims are attached as Appendix B.

In the present Office Action, the pending claims were rejected, in various combinations, under 35 U.S.C. § 112, second paragraph, 35 U.S.C. § 112, first paragraph, under 35 U.S.C. § 102(b) and under 35 U.S.C. § 103(a). Each of these rejections is addressed in turn below in the order set forth by the Examiner.

Objection to the Specification

The Examiner has objected to the specification because page 1, lines 19-20 allegedly contains embedded hyperlinks. Applicants have amended the specification to delete the embedded hyperlink. Accordingly, Applicants respectfully request withdrawal of this objection.

Rejection under 35 U.S.C. § 112, Second Paragraph

Claims 1-3, 14-25, and 30-38 stand rejected under 35 U.S.C. § 112, second paragraph, as allegedly indefinite. Applicants respectfully traverse this rejection.

As set forth in MPEP § 2173.02, "[d]efiniteness of claim language, must be analyzed in light of (A) content of the application; (B) the teachings of the prior art; and (C) the claim interpretation that would be given by one possessing the ordinary level of skill in the pertinent art at the time the invention was made."

Applicants respectfully assert that the specification adequately defines the terms or the terms are adequately understood to one of skill in the art, such that the claims are not indefinite under 35 U.S.C. §112, second paragraph.

Claims 1-3, 14-25, and 30-38 stand rejected under 35 U.S.C. § 112, second paragraph, as allegedly indefinite for the recitation of "in a position proximate." In making the rejection, the Examiner alleges that the exact meaning of the term "proximate" is not clear. Applicants respectfully assert that the term "proximate" is defined in the specification at page 5, lines 2-4 which explicitly states that proximate means "positioned so that [the reactive site] can form a covalent bond with the pendant functional group of the chelate." Thus, one of skill in the art would understand the metes and bounds for the term "proximate" from a reading of the specification.

Claims 18-19 also stand rejected under 35 U.S.C. § 112, second paragraph, as allegedly indefinite for the recitation of "L is a chemical bond or linking group that may contain one or more sites." In accordance with the Examiner's suggestion, Applicants have amended claim 18 to delete the recitation "that may contain one or more sites."

In view of the foregoing remarks and amendments, Applicants respectfully request withdrawal of the rejections under 35 U.S.C. § 112, second paragraph.

Rejections under 35 U.S.C. § 112, First Paragraph

Claims 1-3, 14-25, and 30-38

Claims 1-3, 14-25, and 30-38 stand rejected under 35 U.S.C. § 112, first paragraph, as allegedly non-enabled. In making the rejection, the Examiner states that the specification is enabling for a mutant antibody that comprises a reactive site not present in the wild type parent antibody wherein the mutant antibody comprises six CDRs and specifically binds to a metal chelate wherein the reactive site is in a position proximate to or within a CDR, but alleges that the specification does not reasonably provide enablement for a mutant antibody that does not comprise a full set of six CDRs. Applicants respectfully traverse this rejection.

A particular claim is enabled by the disclosure in an application if the disclosure, at the time of filing, contains sufficient information so as to enable one of skill in the art to make and use the claimed invention without ***undue*** experimentation. *See, e.g., In re Wands*, 8 USPQ2d, 1400 (Fed. Cir. 1988), or MPEP §2164.01. The fact that experimentation may be complex does not necessarily make it undue, if the art typically engages in such experimentation. *See*, MPEP § 2164.01. The test is not merely quantitative, since a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed. MPEP § 2164.06, citing *In re Wands*, 8 USPQ2d, 1400 (Fed. Cir. 1988). As set forth in MPEP § 2164.08, a rejection for undue breadth is inappropriate where "one of skill could readily determine any one of the claimed embodiments."

As defined in the specification at page 11, lines 20-22, a CDR refers to the part of the antibody that recognizes the target or portions thereof. It is known to those of skill in the art that the portions of antibodies, *e.g.*, the variable region of an antibody, are responsible for target recognition (*see, e.g.*, FUNDAMENTAL IMMUNOLOGY, Paul ed. 4th ed. 1999, page 39, col. 2, lines 25 and 26, copy enclosed).

In addition, the specification provides ample guidance to one of skill in the art for making antibody fragments (*see, e.g.*, page 13, line 33 to page 14, line 11, page 15, lines 11-17, page 16, line 24 to page 18, line 2). For example, the specification explicitly states that antibody fragments, including $F(ab')_2$ fragments, Fab monomers, Fv fragments and scFv fragments, can be prepared by modification of whole antibodies or by *de novo* synthesis using recombinant DNA methodology. In addition, the specification provides extensive guidance regarding methods of mutagenizing antibodies that bind to metal chelates to generate mutant antibodies as disclosed and claimed in the present invention (*see, e.g.*, page 23, line 14 to page 39, line 8). Moreover, the specification provides working examples describing the use of rational computer-aided design to develop a mutant antibody that comprises a reactive site not present in the wild type antibody and a CDR that recognizes a metal chelate (*see, e.g.*, page 71, line 1 to page 75, line 5).

Finally, the specification provides a specific working example describing an assay that allows one of skill in the art to readily determine whether a mutation in an antibody has affected the antigen-antibody binding interaction (*see, e.g.*, page 73, line 5 to page 75, line 6).

In making this rejection, the Examiner cites Rudikoff *et al., Proc. Nat'l. Acad. Sci. USA*, 79:1979 (1982), to support the allegation that minor changes in the amino acid sequences of the heavy and light variable regions affect antigen binding and to support the conclusion that the claims are not enabled. Applicants, respectfully assert that the Examiner has mischaracterized the disclosure of Rudikoff *et al.* Contrary to the Examiner's assertion, Rudikoff *et al.* teaches that most substitutions in antibodies do not affect antigen binding. First, Rudikoff *et al.* states that amino acid substitutions in antibodies "may in ***some*** situations be effective in altering antigen-binding specificity" (emphasis in original) (*see*, abstract, lines 15-18). Second, Rudikoff *et al.* explicitly states that "it is clear that all such substitutions need not and probably do not affect antigen binding" (*see*, page 1982, col. 1, lines 25-26). Finally, Rudikoff *et al.* further states that "as many as eight or nine substitutions may occur in hypervariable regions with no significant effect on hapten affinity or specificity" (*see*, page 1982, col. 1, line 31 to col. 2, line 2). Thus Rudikoff *et al.* supports the proposition that amino acid substitutions may be made in antibodies without affecting antigen binding.

Applicants respectfully submit that, based on the guidance and working examples in the specification and what is known to those of skill in the art, one of skill in the art would be able to make and use the claimed mutant antibody comprising a reactive site not present in the wild-type of said antibody and a complementarity-determining region (CDR) that recognizes a metal chelate or portions thereof, wherein said reactive site is in a position proximate to or within said complementarity-determining region.

Claim 14

Claim 14 also stands rejected under 35 U.S.C. § 112, first paragraph, as being allegedly non-enabled. The Examiner states that the specification does not provide evidence that the claimed biological materials are (1) known and readily available to the

public) or (2) reproducible from the written description. In making the rejection, the Examiner states that a deposit of the hybridoma would satisfy the enablement requirements of 35 U.S.C. § 112, first paragraph. Claim 14 recites ""wherein said mutant antibody is a mutant of CHA255."

A deposit of the cell line expressing the S95C antibody, a mutant of the CHA255 antibody, is being deposited with the ATCC in accordance with the provisions of the Budapest Treaty. Once the deposit has been made and the ATCC designation number has been received, the Applicants will submit a true copy of the completed deposit form, amend the specification to recite that date that the deposit was made and the name and address of the depository, provide a verified statement that the deposited material is identical to the biological material described in the specification as filed, and provide a statement from the assignee, the Regents of the University of California, confirming that all restrictions imposed by Regents of the University of California on the availability to the public of the aforementioned deposited material will be irrevocably removed upon the granting of a patent for the above-referenced patent application. Applicants respectfully request that the Examiner hold this rejection in abeyance until such time as the deposit is made and the ATCC designation number has been received.

In view of the foregoing amendments and remarks, Applicants respectfully request withdrawal of the rejections under 35 U.S.C. § 112, first paragraph.

Rejection Under 35 U.S.C. § 102(b)

Claims 1-3, 14, 16-19, and 24 stand rejected under 35 U.S.C. § 102(b) as allegedly anticipated by Stickney *et al.*, *Immunology* 79:1979-1983 (1982). Applicants respectfully traverse this rejection.

For a rejection of claims under § 102(b) to be properly founded, the Examiner must establish that a single prior art reference discloses each and every element of the claimed invention. *See, e.g.*, *Hybritech Inc. v. Monoclonal Antibodies, Inc.*, 231 U.S.P.Q. 81 (Fed. Cir. 1986), *cert. denied*, 480 U.S. 947 (1987). In *Scripps Clinic & Research Found. v. Genentech, Inc.*, 18 U.S.P.Q.2d 1001 (Fed. Cir. 1991), the Federal

Circuit held that "anticipation requires that all of the elements and limitations of the claim are found with a single prior art reference. . . . ***There must be no difference between the claimed invention and the reference disclosure, as viewed by a person of ordinary skill in the field of the invention.***" *Id.* at 1010 (emphasis added). Anticipation can be found, therefore, only when a cited reference discloses ***all*** of the elements, features or limitations of the presently claimed invention.

In making this rejection, the Examiner has alleged that Stickney *et al.* teaches (1) a bifunctional CHA255 antibody which binds a metal chelate and a cell surface antigen; and (2) a reactive SH group not present in the wild type antibody.

Stickney *et al.* describes a $(Fab')_2$ bifunctional antibody coupled by a stable thioether linkage (*see, e.g.*, page 6650, col. 2, lines 7-9, Figure 1, legend, and abstract, line 10-12).

The present invention is directed to a mutant antibody comprising: (1) a reactive site (not a stable linkage) not present on the wild type antibody, and (2) a CDR that specifically binds to a metal chelate. In contrast to the presently claimed mutant antibody, the antibody of Stickney *et al.* does not have ***any*** reactive site. Thus, Stickney *et al.* does not disclose an antibody having a reactive site that is a free sulfhydryl group as disclosed and claimed in the present invention. Thus, an element of the presently claimed invention is absent from the disclosure of Stickney *et al.*

In view of the foregoing, Applicants respectfully submit that since Stickney *et al.*, does not disclose ***all*** of the elements, features or limitations of the presently claimed invention, Stickney *et al.*, cannot form the proper basis for a § 102(b) rejection and respectfully request withdrawal of this rejection.

Rejections under 35 U.S.C. § 103(a)

Claims 1-3, 14, 16-20, 22-23, 25, 30-34, 37, and 38 stand rejected under 35 U.S.C. § 103(a) as allegedly unpatentable over Reardan *et al.*, *Nature* 316:265-267 (1985) and further in view of Orlandi *et al.*, *Proc. Nat'l. Acad. Sci. USA* 86:3833-3837

(1989), Pastan *et al.* (U.S. Patent No. 5,747,654), and Goodwin *et al.*, *J. Nucl. Med.* 29:226-234 (1988). Applicants respectfully traverse this rejection.

As set forth in M.P.E.P. § 2143, "[t]o establish a *prima facie* case of obviousness, *three* basic criteria must be met. *First*, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. *Second*, there must be a reasonable expectation of success. *Finally*, the prior art reference (or references when combined) must teach or suggest all the claim limitations. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, not in applicant's disclosure. *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991)."

All three elements set forth above must be present in order to establish a *prima facie* case of obviousness. As explained herein below, Applicants assert that a *prima facie* case of obviousness has not been established because the cited references do not teach or suggest all the claim limitations. Moreover, one of skill in the art would have no motivation to combine the cited references. Finally, even if the disclosures of the cited references were combined, the combination would not lead to the presently claimed invention.

In making this rejection, the Examiner alleges that Reardan *et al.* teaches antibodies to metal chelates, specifically the CHA255 antibody and a hybridoma that produces that antibody, but acknowledges that Reardan *et al.* does not teach a mutant antibody comprising a reactive site that is not in the wild-type antibody or a bispecific antibody to a cell surface antigen. The Examiner alleges that Orlandi et al, Pastan *et al.*, and Goodwin *et al.* remedy the deficiency in Reardan *et al.* Specifically, the Examiner alleges that Orlandi *et al.* teach a method of cloning the variable domains of an antibody from the hybridoma that produces the antibody; that Pastan *et al.* teach a method of stabilizing an antibody by producing an antibody that has a cysteine group at a position proximate to a CDR; and that Goodwin *et al.* teach antibodies directed to ***metal chelates containing reactive groups***, specifically antibodies to a polyaminocarboxylate of Co(III),

having a reactive SH or acrylamido moiety on the chelate. The Examiner concludes that it would have been obvious to use a metal chelate antibody of Reardan *et al.* and Goodwin *et al.* and protein sequence of the V_H and V_L as taught by Orlandi *et al.* to produce a disulfide stabilized antibody as taught by Pastan *et al.*

The Combination of References Fails to Disclose Each Element of the Applicant's Claimed Invention

As explained above, the present invention is directed to a mutant antibody comprising: (1) a reactive site not present on the wild type antibody, and (2) a CDR that recognizes a metal chelate. The reactive site is in a position proximate to or within the complementarity determining region. Applicants respectfully assert that the combination of the references does not disclose or suggest all of the elements of the present invention.

Reardan *et al.* discloses generation of monoclonal antibodies specific for the EDTA chelate of indium. As acknowledged by the Examiner, Reardan *et al.* contains no suggestion of a mutant antibody comprising a reactive site that is not in the wild-type antibody or a bispecific antibody to a cell surface antigen.

Orlandi *et al.* discloses amplifying the variable regions of antibodies. Orlandi *et al.* contains no suggestion of any mutant antibody and, therefore, no suggestion of a mutant antibody that comprises a reactive site not present in the wild type antibody.

Pastan *et al.* discloses stabilized polypeptide molecules comprising a first variable region of a ligand binding moiety bound through a disulfide bond to a second separate variable region of the ligand binding moiety (*see, e.g.*, col. 1, lines 61-66 and col. 2, lines 12). Pastan *et al.* contains no suggestion that the disulfide ***stabilized*** antibody comprises a reactive group.

Goodwin *et al.* discloses monoclonal antibodies that bind to a 1,4, dithiol spacer group (*see, e.g.*, page 228, col. 2, lines 1-2) of ***a metal chelate***. Contrary to the Examiner's allegation, Goodwin *et al.* contains no mention or suggestion of ***any*** reactive site on an antibody. In addition, Goodwin *et al.* is devoid of any mention or suggestion of any mutant antibody, much less a mutant antibody comprising a reactive site that is not

in the wild-type antibody. In further contrast to the presently claimed mutant antibody, the antibody of Goodwin does not bind to a metal chelate comprising a reactive functional group of complementary reactivity to the reactive site of said antibody.

Thus, an element of the claimed invention is absent from each of the cited references. Specifically, none of the cited references mentions or suggests a mutant antibody that comprises a reactive site not present on the wild type antibody. In the absence of a disclosure or suggestion of each claimed element, a proper *prima facie* case of obviousness has not been set forth.

One Of Skill In The Art Would Have No Motivation To Combine The Cited References

The rejection points to a statement in Reardan *et al.* that "the extension of the present work to prepare monoclonal antibodies with dual antigen specificity, which could simultaneously bind a metal chelate and a physiological antigen" as motivation for combining the disclosures of all the cited references. However, Reardan *et al.* contains no mention or suggestion of a mutant antibody having a reactive site not present on the wild-type antibody as disclosed and claimed in the present invention. Thus, only the application of improper hindsight would lead to a skilled artisan to combine Reardan *et al.*, Orlandi *et al.*, Pastan *et al.*, and Goodwin *et al.* None of the cited references provide any catalyst that would motivate one of skill in the art to combine the references. In the absence of any mention or suggestion that the methods may be combined, the skilled artisan would not be motivated to make such a combination.

Even if the cited references were combined, the combination would not lead to the presently claimed mutant antibody comprising a reactive site not present on the wild type antibody and a CDR that recognizes a metal chelate because, as discussed in detail above, elements of the presently claimed antibody are absent from ***all*** of the disclosures of Reardan *et al.*, Orlandi *et al.*, Pastan *et al.*, and Goodwin *et al.* Specifically, none of the cited references discloses or suggests a mutant antibody having a reactive group not present on the wild-type antibody as disclosed and claimed in the

present invention. Thus, the combination of the cited references would not lead to the presently claimed mutant antibody comprising a reactive site not present on the wild type antibody.

One Of Skill In The Art Would Have No Reasonable Expectation of Success in Producing the Claimed Antibody by Modifying the Cited References

One of skill in the art would have no reasonable expectation of success in modifying the disclosures of the references to produce the claimed mutant antibody having a reactive site not present in the wild-type of the antibody and a CDR that recognizes a metal chelate, wherein the reactive site is in a position proximate to or within the complementarity determining region. There is no suggestion in the cited art that the reactive group could be placed in a location that would allow it to react with a reactive group on a metal chelate bound by the antibody. The cited art provides no guidance regarding any reactive site and, therefore, provides no guidance for placement of a reactive site. The only guidance regarding placement of the reactive site comes from the specification of the present application. Without the explicit guidance in the specification of the present application regarding a mutant antibody having a reactive site not present on the wild-type antibody, wherein the reactive site is in a position proximate to or within the complementarity determining region, one of skill in the art would not have expected that modifying the cited references would successfully produce such an antibody.

The Rejection Of Claims 17 And 31 Is Based On An Erroneous Interpretation That A Targeting Moiety Is The Same As An Antibody

In making this rejection, the Examiner further states that claims 17 and 31 are interpreted to mean that the targeting moiety can be the same as the antibody and alleges that the cited art reads on claims 17 and 31. Applicants respectfully disagree. First, Applicants note that claims 17 and 31 recite that the mutant antibody further comprises a targeting moiety covalently attached thereto. Thus, the claim language indicates that the antibody and the targeting moiety are not the same. Applicants also

note that that the specification explicitly describes the preparation of mutant antibody-targeting moiety conjugates (*see, e.g.*, page 50 line 11 to page 52, line 15). Thus, it is clear from the claims and the specification that the targeting moiety is not the same as the antibody. Since the Examiner's basis for rejecting claims 17 and 31 appears to be based on the interpretation that the targeting moiety is the same as the antibody, withdrawal of this aspect of the rejection is respectfully requested.

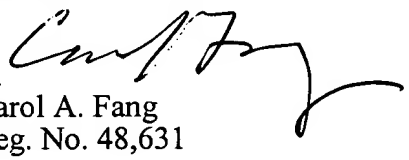
In view of the foregoing remarks, Applicants respectfully submit that the present invention is non-obvious and patentable over Reardan *et al.*, further in view of Orlandi *et al.*, Pastan *et al.*, and Goodwin *et al.* Accordingly, Applicants urge the Examiner to withdraw this rejection under 35 U.S.C. § 103(a).

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at .

Respectfully submitted,



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APPENDIX A
VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE SPECIFICATION

On page 1, lines 17-27, please insert the following replacement paragraph:

Over a million new cases of cancer will be diagnosed this year in the United States (see, for example, American Cancer Society; [http://www.cancer.org/statistics/cff98/basicfact_toc.html] http://www.cancer.org/statistics/cff98/basicfact_toc.html; National Cancer Institute; [http://rex.nci.nih.gov/NCI_Pub_Interface/raterisk/ratestoc.html] http://rex.nci.nih.gov/NCI_Pub_Interface/raterisk/ratestoc.html). While surgery can often provide definitive treatment of cancer in its early stages, the eradication of metastases is crucial to the cure of more advanced disease. Chemotherapeutic drugs are used in combinations for this purpose, with considerable success. Nonetheless, over half a million Americans will die from cancer this year. Progressions and relapses following surgery and chemotherapy/radiation are not uncommon, and in most cases the second line of treatment is of limited use. Despite the expenditure of large amounts of public and private resources over many years, better treatments for cancer are sorely needed.

IN THE CLAIMS

1. A mutant antibody comprising a reactive site not present in the wild-type of said antibody and a complementarity-determining region (CDR) that [specifically binds to] recognizes a metal chelate or portions thereof, wherein said reactive site is in a position proximate to or within said complementarity-determining region.

10. (Once amended) A polypeptide comprising a peptide sequence according to SEQ. ID NO.:5 [**(FIG.11)**] **(FIG. 12)**.

18. (Once amended) The mutant antibody according to claim 17, having the structure:

Ab-L-T

wherein,

Ab represents said antibody;

L is a chemical bond or linking group[that may contain one or more sites]; and

T is said targeting moiety.

25. (Once amended) A mutant antibody comprising a cysteine residue not present in the wild-type of said antibody and a complementarity-determining region that [specifically binds to] recognizes a metal chelate or portions thereof, wherein said cysteine is in a position proximate to or within said complementarity-determining region.

[**39.** (Once amended) A method of treating a patient by administration of a metal chelate, said method comprising the steps of:

(a) administering to said patient a pretargeting reagent;

(b) following step (a), administering to said patient a mutant antibody comprising;

(i) a complementarity-determining region that specifically binds to said metal chelate;

(ii) a reactive site not present in the wild-type of said antibody and, wherein said reactive site is in a position proximate to or within said complementarity-determining region; and

(iii) a recognition moiety that binds specifically with said pretargeting moiety, thereby forming a complex between said pretargeting reagent and said mutant antibody; and

(c) following step (b), administering to said patient said metal chelate, wherein said chelate comprises a reactive functional group having a reactivity complementary to the reactivity of said reactive site of said antibody, thereby;

(i) specifically binding said chelate to said complementarity-determining region; and

(ii) following step (i), forming a covalent bond between said mutant antibody and said metal chelate through coupling the reactive functional group of said chelate with said reactive site of said mutant antibody.]

[**40.** The method according to claim **39**, further comprising, between steps (a) and (b), administering a clearing agent to said patient.]

[**41.** A method of treating a patient by administration of a metal chelate, said method comprising the steps of:

(a) administering to said patient a mutant antibody comprising;

(i) a complementarity-determining region that specifically binds to said metal chelate;

(ii) a reactive site not present in the wild-type of said antibody and, wherein said reactive site is in a position proximate to or within said complementarity-determining region; and

(iii) a recognition moiety that binds specifically with said pretargeting moiety, thereby forming a complex between said pretargeting reagent and said mutant antibody; and

(b) following step (a) administering to said patient said metal chelate, wherein said chelate comprises a reactive functional group having a reactivity complementary to the reactivity of said reactive site of said antibody, thereby;

(i) specifically binding said chelate to said complementarity-determining region; and

(ii) following step (i) forming a covalent bond between said mutant antibody and said metal chelate through coupling the reactive functional group of said chelate with said reactive site of said mutant antibody.]

42. (New) A mutant antibody comprising a reactive site not present in the wild-type of said antibody and a complementarity-determining region (CDR) that specifically binds a metal chelate, wherein said reactive site is in a position proximate to or within said complementarity-determining region.

43. (New) A mutant antibody comprising a reactive site not present in the wild-type of said antibody and a complementarity-determining region (CDR) that recognizes a metal chelate comprising a reactive group or portions thereof, wherein said reactive site is in a position proximate to or within said complementarity-determining region, and

wherein said reactive group has complementary reactivity to said reactive site of said antibody.

FUNDAMENTAL IMMUNOLOGY

FOURTH EDITION

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APPENDIX C

Each individual polypeptide chain consists of two to five domains of approximately 110 amino acids (7), each capable of folding independently. These domains form compact, protease-resistant structures which serve as the fundamental unit of immunoglobulin structure. The interactions that allow for the formation of the aforementioned immunoglobulin monomer almost exclusively occur in pair-wise fashion between domains of two different polypeptide chains (see Fig. 1), such that the functional modules of an antibody are in fact dimerized domains. In addition, as each domain of an antibody molecule is encoded by a separate exon, immunoglobulin domains also serve as the essential element of antibody genetics. In this light, it is easy to recognize how evolution has used the prototypical immunoglobulin domain as a substrate for experimentation, and as a result different domains have attained distinct structural and functional attributes. Moreover, the presence of one or more "immunoglobulin homology domains" also proves to be the distinguishing characteristic for inclusion in the immunoglobulin gene superfamily. Thus, the duplication and adaptation of the Ig homology domain has occurred not only within the context of formal "immunoglobulin genes," but also in the greater scope of the IgSF, which far predates the emergence of antibody. In either case, the archetypal immunoglobulin domain has clearly proven to be a powerful evolutionary tool, as will be detailed below and in greater detail throughout this chapter.

The hallmark of all Ig domains is the presence of a structural motif termed the *immunoglobulin fold.* This characteristic feature is actually a specialized "β-barrel" typically comprised of seven polypeptide strands, which form antiparallel β-pleated sheets in the folded domain. This configuration is depicted in Fig. 2, which was deduced from x-ray diffraction studies of an immunoglobulin light chain (8). Each Ig domain is composed of two β-pleated sheets, one containing four β strands, the other consisting of at least three β strands (represented by arrows in Fig. 2). Loops of variable length connect the different strands, allowing the β sheets to form. The two β-pleated layers are oriented in a sandwich, enclosing a hydrophobic interior. Further stability is provided by a disulfide bond near the domain's core, which covalently links the two sheet layers. The cysteines that contribute this bond are conserved in all immunoglobulins, and in almost all proteins that possess Ig-like domains. Two residues, a tryptophan in strand 3-1 and an aromatic residue that precedes the second half-cystine, are also maintained consistently and serve to protect the disulfide bond in the three-dimensional structure. Other conserved features include hydrophobic core residues, which stabilize the inside of the sandwich, and glycine and proline loop residues, which provide the flexibility necessary for the formation of these interconnecting sequences (9–12).

Since the hydrophobic core residues are predominantly responsible for promoting the folding of the β sheets, and thus the entire immunoglobulin fold, the sequences of the loop residues are free to vary considerably. This, in turn, grants loop residues the freedom to serve as substrates for selection, at the level of selection of a particular antibody in an immune response and at the level of natural selection in phylogeny. In this way, the prototypical immunoglobulin homology domain serves as a potent cofactor for the evolution of both organismal immunity and that of the species in general.

Immunoglobulin Nomenclature

Light chains contain two such immunoglobulin domains, whereas a heavy chain is made up of either four or five domains, depending on the type of heavy chain (isotype) used by the antibody in question. Different immunoglobulin domains possess different structural and functional characteristics, and their naming, in part, reflects these differences. The amino-terminal domain of each chain, whether of the heavy or light type, is termed a *variable* (V) region due to the discovery of extensive sequence divergence between different antibody proteins in this part of the molecule. These are designated V_H and V_L for heavy and light chains, respectively. V regions have been demonstrated to be responsible for the antigenic specificity of the immunoglobulin.

Carboxy-terminal domains, on the other hand, display considerably less sequence variation within a given isotype and are referred to as *constant* (C) regions. Heavy chain C regions are numbered C_H1, C_H2, and so on, beginning with the most V region-proximal domain. The constant region domains of the heavy chain have been shown to be responsible for many aspects of antibody function, including interaction with Fc receptors, complement fixation, transplacental transfer, the ability to multimerize, and the capacity to be secreted on mucosal surfaces. Because different heavy chain isotypes have different C region domains (i.e., the C_H3 domains of different isotypes are distinct), these capabilities vary with the class of the particular antibody. Five major classes of heavy chain C regions exist: alpha (α), gamma (γ), delta (δ), epsilon (ε), and mu (μ). As a direct consequence of the correlation between the

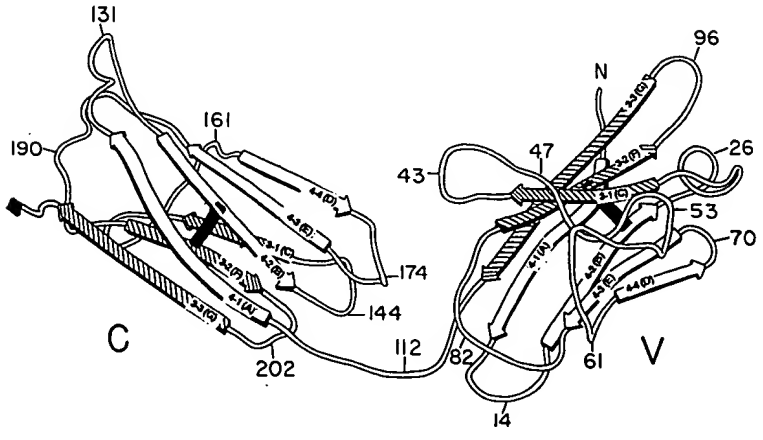

FIG. 2. Ribbon drawing of the V and C domains of a light chain. β strands are depicted as *arrows,* with those of the four-stranded face *unshaded* and those of the three-stranded face *shaded.* Strands are numbered according to Edmundson and lettered (*in parentheses*) according to Hood. Intrachain disulfide bonds are represented as *black bars.* Selected amino acids are numbered, with position 1 being the N-terminus. Residues 26, 53, and 96 correspond to amino acids in CDRs 1, 2, and 3, respectively. The dimerization surfaces of each domain (four-strand side of the C domain, three-strand side of the V domain) face upwards. (Adapted from ref 8, with permission.)